

1 CLAIMS

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3 We claim:

4  
5 1. A method for producing a decellularized tissue engineered construct comprising the steps of:  
6 providing a tissue engineered construct; and  
7 decellularizing the tissue engineered construct, thereby forming a decellularized tissue  
8 engineered construct.  
9

10 2. The method of claim 1, wherein the providing step comprises producing a tissue engineered  
11 construct, and wherein producing a tissue engineered construct comprises the steps of:  
12 contacting a substrate with a population of cells capable of adhering thereto, thereby  
13 forming a cell-seeded construct; and  
14 maintaining the cell-seeded construct under conditions suitable for growth of the  
15 population of cells for a growth period to form a tissue engineered construct.  
16

17 3. The method of claim 1, wherein the providing step comprises producing a tissue engineered  
18 construct, and wherein producing a tissue engineered construct comprises the steps of:  
19 contacting a substrate with a first population of cells capable of adhering thereto, thereby  
20 forming a primary cell-seeded construct; and  
21 maintaining the cell-seeded construct under conditions suitable for growth of the first  
22 population of cells for a first growth period to form a primary tissue engineered construct;  
23 contacting the primary tissue engineered construct with a second population of cells,  
24 thereby forming a secondary cell-seeded construct; and  
25 maintaining the secondary cell-seeded construct under conditions suitable for growth of  
26 the second population of cells for a second growth period.  
27

28 4. The method of claim 2, wherein the contacting and maintaining steps are repeated alternately  
29 until a cell-seeded construct having desired properties is formed.

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2 5. The method of claim 4, wherein the contacting step is repeated using a plurality of different  
3 cell types.

4  
5 6. The method of claim 2, wherein the substrate comprises a biocompatible material.

6  
7 7. The method of claim 2, wherein the substrate comprises a porous material.

8  
9 8. The method of claim 2, wherein the substrate comprises a collagen sponge.

10  
11 9. The method of claim 2, wherein the substrate comprises a polymeric material.

12  
13 10. The method of claim 2, wherein the substrate comprises a length of tubing.

14  
15 11. The method of claim 10, wherein the length of tubing is coated.

16  
17 12. The method of claim 2, wherein the substrate comprises a synthetic polymeric material.

18  
19 13. The method of claim 12, wherein the synthetic polymeric material has a hydrophilic surface.

20  
21 14. The method of claim 12, wherein the polymeric material comprises a polymer selected from  
22 the group consisting of polyesters of hydroxycarboxylic acids, polyanhydrides of dicarboxylic  
23 acids, and copolymers of hydroxy carboxylic acids and dicarboxylic acids.

24  
25 15. The method of claim 2 wherein the substrate has an inner and outer surface, wherein the  
26 inner surface of the substrate defines a lumen.

27  
28 16. The method of claim 2 wherein the substrate comprises a flat surface.

29  
30 17. The method of claim 2 wherein the substrate comprises a three-dimensional structure.

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2 18. The method of claim 2, wherein a mechanical force is applied to the construct during the  
3 growth period.

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5 19. The method of claim 2, wherein a pulsatile stimulus is applied to the construct during the  
6 growth period.

7  
8 20. The method of claim 2, wherein pulsatile stretch is applied to the construct during the growth  
9 period.

10  
11 21. The method of claim 2, wherein the growth period is continued until the construct reaches a  
12 predetermined thickness.

13  
14 22. The method of claim 2, wherein the growth conditions are chosen to promote deposition of  
15 extracellular matrix components.

16  
17 23. The method of claim 2, wherein the cells are selected from the group consisting of: smooth  
18 muscle cells, cardiac muscle cells, epithelial cells, endothelial cells, urothelial cells, fibroblasts,  
19 myoblasts, chondrocytes, chondroblasts, osteoblasts, osteoclasts, hepatocytes, bile duct cells,  
20 pancreatic islet cells, thyroid, parathyroid, adrenal, hypothalamic, pituitary, ovarian, testicular,  
21 salivary gland cells, adipocytes, and precursor cells.

22  
23 24. The method of claim 2, wherein the cells are neonatal cells.

24  
25 25. The method of claim 2, wherein the population of cells comprises cells of at least two cell  
26 types.

27  
28 26. The method of claim 2, wherein the cells are human cells.

29  
30 27. The method of claim 2, wherein the cells are porcine cells.

1  
2 28. The method of claim 2, wherein the cells are tumor cells.

3  
4 29. The method of claim 2, wherein the cells are genetically transformed cells.

5  
6 30. The method of claim 1 or 2, wherein the decellularization step comprises:  
7 incubating the construct in a processing solution, the processing solution extracting cells  
8 from the construct.

9  
10 31. The method of claim 30, wherein the processing solution comprises at least one component  
11 selected from the list consisting of: a detergent, a hypotonic solution, an RNA nuclease, and a  
12 DNA nuclease.

13  
14 32. The method of claim 1 or 2, wherein at least 50% of the cells are removed in the  
15 decellularization step.

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17 33. The method of claim 1 or 2, wherein at least 60% of the cells are removed in the  
18 decellularization step.

19  
20 34. The method of claim 1 or 2, wherein at least 70% of the cells are removed in the  
21 decellularization step.

22  
23 35. The method of claim 1 or 2, wherein at least 80% of the cells are removed in the  
24 decellularization step.

25  
26 36. The method of claim 1 or 2, wherein at least 90% of the cells are removed in the  
27 decellularization step.

28  
29 37. The method of claim 1 or 2, wherein at least 95% of the cells are removed in the  
30 decellularization step.

1  
2 38. The method of claim 1 or 2, wherein at least 99% of the cells are removed in the  
3 decellularization step.

4  
5 39. The method of claim 1 or 2, wherein substantially all of the cells are removed in the  
6 decellularization step.

7  
8 40. The method of claim 2, further comprising the step of:  
9 removing a portion of the substrate.

10  
11 41. The method of claim 2, further comprising the step of:  
12 removing substantially all of the substrate.

13  
14 42. The method of claim 2, further comprising the step of:  
15 applying a fluid shear to the decellularized construct, thereby removing a portion of the  
16 substrate.

17  
18 43. The method of claim 2, further comprising the step of:  
19 applying a fluid shear to the decellularized construct, thereby removing substantially all  
20 of the substrate.

21  
22 44. The method of claim 2, further comprising the step of:  
23 storing the decellularized tissue engineered construct.

24  
25 45. The method of claim 44, further comprising the step of:  
26 before storing the decellularized construct, pretreating the decellularized construct with  
27 an agent selected to protect the decellularized construct during the storage process.

28  
29 46. The method of claim 44, wherein the storing comprises cryopreservation.  
30

1 47. The method of claim 46, wherein the decellularized construct comprises a proteinaceous  
2 matrix, and wherein the storing step comprises:  
3 incubating the construct in a cryoprotective solution and freezing at cooling rates such  
4 that minimal functional damage occurs to the proteinaceous matrix of the construct to produce a  
5 cryoprepared construct;  
6 drying the cryoprepared construct under temperature and pressure conditions that permit  
7 removal of water without substantial ice recrystallization or ultrastructural damage.  
8

9 48. The method of claim 44, wherein the storing comprises drying.  
10

11 49. The method of claim 44, further comprising the step of:  
12 reconstituting the decellularized construct after storage.  
13

14 50. The method of claim 49, wherein the reconstituting step comprises:  
15 incubating the decellularized construct in a rehydration solution, the rehydration solution  
16 reducing osmotic, hypoxic, autolytic, or proteolytic damage.  
17

18 51. The method of claim 49, wherein the reconstituting step comprises:  
19 incubating the decellularized construct in a rehydration solution, the rehydration solution  
20 reducing microbial contamination.  
21

22 52. The method of claim 44, further comprising the step of:  
23 treating the decellularized construct with a biologically active agent.  
24

25 53. The method of claim 52, wherein the biologically active agent is selected to stimulate  
26 recellularization of the construct.  
27

28 54. The method of claim 52, wherein the biologically active agent is selected from the group  
29 consisting of: growth factors, adhesion factors, soluble extracellular matrix proteins,  
30 thrombomodulators, antibiotics, and agents that augment hemocompatibility.

1  
2 55. The method of claim 1 or 2, further comprising the step of:

3       subjecting the decellularized construct to further tissue engineering.  
4

5 56. The method of claim 1, wherein providing a tissue engineered construct comprises:

6       purchasing a tissue engineered construct.  
7

8 57. The method of claim 1, wherein providing a tissue engineered construct comprises providing  
9 a tissue engineered construct that has been produced primarily by growth *in vitro*.  
10

11 58. The method of claim 1, wherein providing a tissue engineered construct comprises providing  
12 a tissue engineered construct that has been produced at least in part by growth *in vivo*.  
13

14 59. A method for treating a subject suffering from tissue damage or loss comprising:

15       producing a decellularized construct according to the method of claim 1 or 2; and

16       implanting the decellularized construct into a subject in need thereof.  
17

18 60. The method of claim 59, wherein the implanting step comprises supplementing or replacing a  
19 blood vessel of the subject.  
20

21 61. The method of claim 59, wherein the implanting step comprises supplementing or replacing a  
22 tissue of the subject, the tissue selected from the list consisting of: a heart valve, a muscle, a  
23 joint, a ligament, a tendon, a bone, and an organ.  
24

25 62. A method for producing an engineered construct comprising the steps of:

26       producing a tissue engineered construct;

27       decellularizing the tissue engineered construct, thereby forming a decellularized

28 construct;

29       contacting the decellularized construct with cells capable of adhering thereto, thereby

30 forming a cell-seeded decellularized construct; and

1 maintaining the cell-seeded decellularized construct for a growth period in an  
2 environment suitable for growth of the cells to form an engineered construct.

3  
4 63. The method of claim 62, wherein the producing step comprises:

5 contacting a substrate with a population of cells capable of adhering thereto, thereby  
6 forming a cell-seeded construct; and

7 maintaining the cell-seeded construct under conditions suitable for growth of the  
8 population of cells for a growth period to form a tissue engineered construct.

9  
10 64. The method of claim 62, wherein the producing step comprises:

11 contacting a substrate with a first population of cells capable of adhering thereto, thereby  
12 forming a primary cell-seeded construct; and

13 maintaining the cell-seeded construct under conditions suitable for growth of the first  
14 population of cells for a first growth period to form a primary tissue engineered construct;

15 contacting the primary tissue engineered construct with a second population of cells,  
16 thereby forming a secondary cell-seeded construct; and

17 maintaining the secondary cell-seeded construct under conditions suitable for growth of  
18 the second population of cells for a second growth period.

19  
20 65. The method of claim 62, wherein the cells comprise human cells.

21  
22 66. The method of claim 62, wherein the cells comprise genetically transformed cells.

23  
24 67. The method of claim 62, wherein the cells are obtained by harvesting cells from a subject, the  
25 subject being the intended recipient of the tissue engineered construct.

26  
27 68. The method of claim 62, wherein the cells are selected from the group consisting of: smooth  
28 muscle cells, cardiac muscle cells, epithelial cells, endothelial cells, urothelial cells, fibroblasts,  
29 myoblasts, chondrocytes, chondroblasts, osteoblasts, osteoclasts, hepatocytes, bile duct cells,



1 pancreatic islet cells, thyroid, parathyroid, adrenal, hypothalamic, pituitary, ovarian, testicular,  
2 salivary gland cells, adipocytes, and precursor cells.

3  
4 69. The method of claim 68, wherein the cells comprise cells of at least two different cell types.

5  
6 70. The method of claim 63 or claim 64, further comprising the step of:  
7 removing a portion of the substrate.

8  
9 71. The method of claim 63 or claim 64, further comprising the step of:  
10 removing substantially all of the substrate.

11  
12 72. The method of claim 63 or claim 64, further comprising the step of:  
13 applying a fluid shear to the decellularized construct, thereby removing a portion of the  
14 substrate.

15  
16 73. The method of claim 63 or claim 64, further comprising the step of:  
17 applying a fluid shear to the decellularized construct, thereby removing substantially all  
18 of the substrate.

19  
20 74. The method of claim 62, further comprising the step of:  
21 after decellularizing the tissue engineered construct to obtain a decellularized construct,  
22 storing the decellularized construct under conditions selected to preserve the construct.

23  
24 75. The method of claim 74, further comprising the step of:  
25 before storing the decellularized construct, pretreating the decellularized construct with  
26 an agent selected to protect the construct during the storage process.

27  
28 76. The method of claim 74, wherein the storing comprises cryopreservation.

29  
30 77. The method of claim 74, wherein the storing comprises drying.

1  
2 78. The method of claim 74, further comprising the step of:

3 reconstituting the decellularized construct after storage.  
4

5 79. The method of claim 78, further comprising the step of:

6 treating the decellularized construct with a biologically active agent.  
7

8 80. The method of claim 79, wherein the biologically active agent is selected to stimulate  
9 recellularization of the construct.  
10

11 81. The method of claim 79, wherein the biologically active agent is selected from the group  
12 consisting of: growth factors, adhesion factors, soluble extracellular matrix proteins,  
13 thrombomodulators, antibiotics, and agents that augment hemocompatibility.  
14

15 82. A method for producing a decellularized engineered native tissue comprising the steps of:  
16 procuring a tissue harvested from an animal or human;  
17 engineering the harvested tissue, thereby forming an engineered native tissue; and  
18 decellularizing the engineered native tissue, thereby forming a decellularized engineered  
19 native tissue.  
20

21 83. The method of claim 82, wherein the engineering step comprises:

22 seeding the harvested native tissue with cells; and

23 maintaining the tissue under conditions suitable for growth of the cells for a growth  
24 period.  
25

26 84. The method of claim 82, wherein the engineering step comprises:

27 subjecting the harvested tissue to a mechanical force, the mechanical force selected to  
28 enhance the properties of the tissue.  
29

30 85. The method of claim 82, wherein the engineering step comprises:

1           subjecting the harvested tissue to an electrical stimulus.

2  
3    86. The method of claim 82, wherein the engineering step comprises:  
4           subjecting the harvested tissue to a pulsatile stimulus.

5  
6    87. The method of claim 82, wherein the engineering step comprises:  
7           treating the harvested tissue with a biologically active agent.

8  
9    88. The method of claim 87, wherein the biologically active agent is selected from the list  
10   consisting of: growth factors, adhesion factors, soluble extracellular matrix proteins,  
11   thrombomodulators, antibiotics, and agents that augment hemocompatibility.

12  
13   89. The method of claim 87, wherein the biologically active agent comprises:  
14           a pharmaceutical composition.

15  
16   90. The method of claim 82, wherein the harvested tissue comprises a blood vessel.

17  
18   91. The method of claim 82, wherein the harvested tissue comprises a heart valve.

19  
20   92. The method of claim 82, wherein the harvested tissue comprises an organ or a portion  
21   thereof.

22  
23   93. The method of claim 82, wherein the harvested tissue comprises a muscle.

24  
25   94. The method of claim 82, further comprising the step of:  
26           subjecting the decellularized engineered native tissue to further tissue engineering.

27  
28   95. The method of claim 82, further comprising the step of:  
29           seeding the decellularized engineered native tissue with cells.

1 96. The method of claim 95 wherein the cells are selected from the group consisting of: smooth  
2 muscle cells, cardiac muscle cells, epithelial cells, endothelial cells, urothelial cells, fibroblasts,  
3 myoblasts, chondrocytes, chondroblasts, osteoblasts, osteoclasts, hepatocytes, bile duct cells,  
4 pancreatic islet cells, thyroid, parathyroid, adrenal, hypothalamic, pituitary, ovarian, testicular,  
5 salivary gland cells, adipocytes, and precursor cells.

6  
7 97. The method of claim 95, wherein the cells comprise cells of at least two different cell types.

8  
9 98. The method of claim 95, wherein the cells comprise neonatal cells.

10  
11 99. The method of claim 95, wherein the cells comprise human cells.

12  
13 100. The method of claim 95, wherein the cells comprise genetically transformed cells.

14  
15 101. A method for treating a subject suffering from tissue damage or loss comprising the steps  
16 of:

17 producing an engineered, decellularized construct according to the method of claim 62;

18 and

19 implanting the tissue engineered construct into a subject in need thereof.

20  
21 102. The method of claim 101, wherein the cells used in the final contacting step are obtained by  
22 harvesting cells from the subject.

23  
24 103. The method of claim 101, wherein the cells used in the final contacting step are obtained by  
25 a method comprising the steps of:

26 harvesting cells from the subject; and

27 culturing the cells *in vitro* prior to seeding the decellularized construct.

28  
29 104. The method of claim 101, wherein the implanting step comprises supplementing or  
30 replacing a blood vessel of the subject.

1

2 105. The method of claim 101, wherein the implanting step comprises supplementing or  
3 replacing a tissue of the subject, the tissue selected from the list consisting of: a heart valve, a  
4 muscle, a joint, a ligament, a tendon, a bone, and an organ.

5

6 106. The method of claim 101, further comprising the step of:  
7 treating the engineered, decellularized construct with a biologically active agent before  
8 the implanting step, whereby the construct serves as a vehicle for delivery of the biologically  
9 active agent to the subject.

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11 107. The method of claim 106, further comprising the step of:

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1           treating the engineered, decellularized construct with a biologically active agent before  
2 the implanting step, wherein the biologically active agent is selected to enhance recellularization  
3 or vascularization of the construct after the implanting step.  
4

5   108. The method of claim 106, wherein the biologically active agent comprises a pharmaceutical  
6 composition.  
7

8   109. The method of claim 106, wherein the biologically active agent is selected from the group  
9 consisting of: growth factors, adhesion factors, soluble extracellular matrix proteins,  
10 thrombomodulators, antibiotics, and agents that augment hemocompatibility.  
11

12   110. An engineered tissue for use as a tissue engineering scaffold or for implanting into a subject  
13 comprising:  
14       a decellularized engineered native tissue.  
15

16   111. The engineered tissue of claim 110, wherein at least 50% of the cells are removed from the  
17 decellularized engineered native tissue by decellularization.  
18

19   112. The engineered tissue of claim 110, wherein at least 60% of the cells are removed from the  
20 decellularized engineered native tissue by decellularization.  
21

22   113. The engineered tissue of claim 110, wherein at least 70% of the cells are removed from the  
23 decellularized engineered native tissue by decellularization.  
24

25   114. The engineered tissue of claim 110, wherein at least 80% of the cells are removed from the  
26 decellularized engineered native tissue by decellularization.  
27

28   115. The engineered tissue of claim 110, wherein at least 90% of the cells are removed from the  
29 decellularized engineered native tissue by decellularization.  
30

1 116. The engineered tissue of claim 110, wherein at least 95% of the cells are removed from the  
2 decellularized engineered native tissue by decellularization.

3  
4 117. The engineered tissue of claim 110, wherein at least 99% of the cells are removed from the  
5 decellularized engineered native tissue by decellularization.

6  
7 118. The engineered tissue of claim 110, further comprising a biologically active agent.

8  
9 119. The engineered tissue of claim 110, wherein the biologically active agent is selected to  
10 enhance recellularization or vascularization of the tissue engineered construct.

11  
12 120. The engineered tissue of claim 110, wherein the biologically active agent comprises a  
13 pharmaceutical composition.

14  
15 121. The engineered tissue of claim 110, wherein the biologically active agent is selected from  
16 the group consisting of: growth factors, adhesion factors, soluble extracellular matrix proteins,  
17 thrombomodulators, antibiotics, and agents that augment hemocompatibility.

18  
19 122. The engineered tissue of claim 110, wherein the engineered native tissue comprises native  
20 tissue that has been subjected to a mechanical force after removal from an animal or human  
21 source, wherein the mechanical force is selected to enhance the properties of the tissue.

22  
23 123. The engineered tissue of claim 110, wherein the engineered native tissue comprises native  
24 tissue that has been subjected to electrical stimulation after removal from an animal or human  
25 source.

26  
27 124. The engineered tissue of claim 110, wherein the engineered native tissue comprises native  
28 tissue that has been treated with a growth factor after removal from an animal or human source.

1 125. The engineered tissue of claim 110, wherein the engineered native tissue comprises native  
2 tissue that has been exposed to serum after removal from an animal or human source.

3  
4 126. The engineered tissue of claim 110, wherein the engineered native tissue comprises native  
5 tissue that has been exposed to a pulsatile stimulus after removal from an animal or human  
6 source.

7  
8 127. The engineered tissue of claim 110, further comprising:  
9 a population of cells, wherein the decellularized engineered native tissue is seeded with  
10 the population of cells after decellularization.

11  
12 128. The engineered tissue of claim 127, wherein the decellularized engineered native tissue is  
13 maintained under conditions suitable for growth of the cells for a growth period following  
14 seeding.

15  
16 129. The engineered tissue of claim 127, wherein the cells comprise human cells.

17  
18 130. The engineered tissue of claim 127, wherein the cells comprise porcine cells.

19  
20 131. The engineered tissue of claim 127, wherein the cells comprise neonatal cells.

21  
22 132. A construct for use as a tissue engineering scaffold or for implanting into a subject  
23 comprising:

24 a tissue engineered construct that has been subjected to decellularization.

25  
26 133. The construct of claim 132, wherein the tissue engineered construct comprises a substrate  
27 seeded with cells and maintained under conditions suitable for growth of the cells for a growth  
28 period.



1 134. The construct of claim 133, wherein the growth period comprises a period of time sufficient  
2 for formation of a tissue engineered construct having a predetermined thickness.

3  
4 135. The construct of claim 133, wherein at least 50% of the cells are removed from the tissue  
5 engineered construct by decellularization.

6  
7 136. The construct of claim 133, wherein at least 60% of the cells are removed from the tissue  
8 engineered construct by decellularization.

9  
10 137. The construct of claim 133, wherein at least 70% of the cells are removed from the tissue  
11 engineered construct by decellularization.

12  
13 138. The construct of claim 133, wherein at least 80% of the cells are removed from the tissue  
14 engineered construct by decellularization.

15  
16 139. The construct of claim 133, wherein at least 90% of the cells are removed from the tissue  
17 engineered construct by decellularization.

18  
19 140. The construct of claim 133, wherein at least 95% of the cells are removed from the tissue  
20 engineered construct by decellularization.

21  
22 141. The construct of claim 133, wherein at least 99% of the cells are removed from the tissue  
23 engineered construct by decellularization.

24  
25 142. The construct of claim 132, further comprising a biologically active agent.

26  
27 143. The construct of claim 132, wherein the biologically active agent is selected to enhance  
28 recellularization or vascularization of the tissue engineered construct.

1 144. The construct of claim 132, wherein the biologically active agent comprises a  
2 pharmaceutical composition.

3  
4 145. The construct of claim 132, wherein the biologically active agent is selected from the group  
5 consisting of: growth factors, adhesion factors, soluble extracellular matrix proteins,  
6 thrombomodulators, antibiotics, and agents that augment hemocompatibility.

7  
8 146. The construct of claim 132, wherein the tissue engineered construct comprises a tissue  
9 engineered construct that has been subjected to a mechanical force during a growth period.

10  
11 147. The construct of claim 132, wherein the tissue engineered construct comprises a tissue  
12 engineered construct that has been subjected to a pulsatile stimulus during a first growth period.

13  
14 148. The construct of claim 132, wherein the tissue engineered construct comprises a tissue  
15 engineered construct that has been subjected to electrical stimulation during a first growth  
16 period.

17  
18 149. The construct of claim 132, wherein the tissue engineered construct comprises a tissue  
19 engineered construct that has been treated with a growth factor during a first growth period.

20  
21 150. The construct of claim 132, wherein the tissue engineered construct comprises a tissue  
22 engineered construct that has been exposed to serum during a first growth period.

23  
24 151. The construct of claim 133, wherein the substrate comprises a polymeric material.

25  
26 152. The construct of claim 133, wherein the substrate comprises a length of tubing.

27  
28 153. The construct of claim 133, wherein the length of tubing is coated.

29  
30 154. The construct of claim 133, wherein the substrate comprises a synthetic polymeric material.

1  
2 155. The construct of claim 133, wherein the polymeric material comprises a polymer selected  
3 from the group consisting of polyesters of hydroxycarboxylic acids, polyanhydrides of  
4 dicarboxylic acids, and copolymers of hydroxy carboxylic acids and dicarboxylic acids.

5  
6 156. The construct of claim 133, wherein the substrate comprises a collagen sponge.

7  
8 157. The construct of claim 133, wherein the substrate has an inner and outer surface, and  
9 wherein the inner surface of the substrate defines a lumen.

10  
11 158. The construct of claim 133, wherein the substrate comprises a flat surface.

12  
13 159. The construct of claim 133, wherein the substrate comprises a three-dimensional structure.

14  
15 160. The construct of claim 133, wherein the construct is treated so as to remove substantially all  
16 of the substrate.

17  
18 161. The construct of claim 133, wherein the cells are selected from the group consisting of:  
19 smooth muscle cells, cardiac muscle cells, epithelial cells, endothelial cells, urothelial cells,  
20 fibroblasts, myoblasts, chondrocytes, chondroblasts, osteoblasts, osteoclasts, hepatocytes, bile  
21 duct cells, pancreatic islet cells, thyroid, parathyroid, adrenal, hypothalamic, pituitary, ovarian,  
22 testicular, salivary gland cells, adipocytes, and precursor cells.

23  
24 162. The construct of claim 133, wherein the cells comprise cells of at least two different cell  
25 types.

26  
27 163. The construct of claim 133, wherein the cells comprise neonatal cells.

28  
29 164. The construct of claim 133, wherein the cells comprise human cells.

1 165. The construct of claim 133, wherein the cells comprise porcine cells.

2

3 166. The construct of claim 133, wherein the cells comprise tumor cells.

4

5 167. The construct of claim 133, wherein the cells comprise genetically transformed cells.

6

7 168. A method for treating a subject suffering from tissue damage or loss comprising:

8       implanting the construct of claim 132 into a subject in need thereof.

9

10 169. The method of claim 168, further comprising the step of:

11       treating the construct with a biologically active agent before the implanting step, whereby

12 the construct serves as a vehicle for delivery of the biologically active agent to the subject.

13

14 170. The method of claim 168, further comprising the step of:

15       treating the construct with a biologically active agent before the implanting step, whereby

16 the biologically active agent is selected to enhance recellularization or vascularization of the  
17 construct after the implanting step.

18

19 171. The method of claim 168, wherein the biologically active agent comprises a pharmaceutical  
20 composition.

21

22 172. The method of claim 168, wherein the biologically active agent is selected from the group  
23 consisting of: growth factors, adhesion factors, soluble extracellular matrix proteins,  
24 thrombomodulators, antibiotics, and agents that augment hemocompatibility.

25

26 173. The method of claim 168, wherein the implanting step comprises supplementing or  
27 replacing a blood vessel of the subject.

28

1 174. The method of claim 168, wherein the implanting step comprises supplementing or  
2 replacing a tissue of the subject, the tissue selected from the list consisting of: a heart valve, a  
3 muscle, a joint, a ligament, a tendon, a bone, and an organ.

4  
5 175. A method for treating a subject suffering from tissue damage or loss comprising:  
6 implanting the engineered tissue of claim 110 into a subject in need thereof.

7  
8 176. The method of claim 175, further comprising the step of:  
9 treating the engineered tissue with a biologically active agent before the implanting step,  
10 whereby the engineered tissue serves as a vehicle for delivery of the biologically active agent to  
11 the subject.

12  
13 177. The method of claim 175, further comprising the step of:  
14 treating the engineered tissue with a biologically active agent before the implanting step,  
15 whereby the biologically active agent is selected to enhance recellularization or vascularization  
16 of the engineered tissue after the implanting step.

17  
18 178. The method of claim 175, wherein the biologically active agent comprises a pharmaceutical  
19 composition.

20  
21 179. The method of claim 175, wherein the biologically active agent is selected from the group  
22 consisting of: growth factors, adhesion factors, soluble extracellular matrix proteins,  
23 thrombomodulators, antibiotics, and agents that augment hemocompatibility.

24  
25 180. The method of claim 175, wherein the implanting step comprises supplementing or  
26 replacing a blood vessel of the subject.

27  
28 181. The method of claim 175, wherein the implanting step comprises supplementing or  
29 replacing a tissue of the subject, the tissue selected from the list consisting of: a heart valve, a  
30 muscle, a joint, a ligament, a tendon, a bone, and an organ.

1  
2 182. A construct for use in tissue engineering or for implanting into a subject comprising:

3 a decellularized tissue engineered construct; and

4 a population of cells, wherein the decellularized tissue engineered construct is seeded  
5 with the population of cells.

6  
7 183. The construct of claim 182, wherein the decellularized tissue engineered construct  
8 comprises a decellularized tissue engineered construct that has been subjected to a mechanical  
9 force during a growth period.

10  
11 184. The construct of claim 182, wherein the decellularized tissue engineered construct  
12 comprises a decellularized tissue engineered construct that has been subjected to a pulsatile  
13 stimulus during a growth period.

14  
15 185. The construct of claim 182, wherein the decellularized tissue engineered construct  
16 comprises a decellularized tissue engineered construct that has been subjected to electrical  
17 stimulation during a growth period.

18  
19 186. The construct of claim 182, wherein the decellularized tissue engineered construct  
20 comprises a decellularized tissue engineered construct that has been treated with a growth factor  
21 during a growth period.

22  
23 187. The construct of claim 182, wherein the decellularized tissue engineered construct  
24 comprises a decellularized tissue engineered construct that has been exposed to serum during a  
25 growth period.

26  
27 188. The construct of claim 182, wherein the decellularized tissue engineered construct  
28 comprises a decellularized tissue engineered construct produced using human cells.

1 189. The construct of claim 182, wherein the decellularized tissue engineered construct  
2 comprises a decellularized tissue engineered construct produced using neonatal cells.

3  
4 190. The construct of claim 182, wherein the decellularized tissue engineered construct  
5 comprises a decellularized tissue engineered construct produced using genetically transformed  
6 cells.

7  
8 191. The construct of claim 182, wherein the decellularized tissue engineered construct  
9 comprises a decellularized tissue engineered construct produced using human cells.

10  
11 192. The construct of claim 182, wherein the decellularized tissue engineered construct  
12 comprises a decellularized tissue engineered construct produced using cells selected from the  
13 group consisting of: smooth muscle cells, cardiac muscle cells, epithelial cells, endothelial cells,  
14 urothelial cells, fibroblasts, myoblasts, chondrocytes, chondroblasts, osteoblasts, osteoclasts,  
15 hepatocytes, bile duct cells, pancreatic islet cells, thyroid, parathyroid, adrenal, hypothalamic,  
16 pituitary, ovarian, testicular, salivary gland cells, adipocytes, and precursor cells.

17  
18 193. The tissue engineered construct of claim 182, wherein the cells comprise cells harvested  
19 from an intended recipient of the construct.

20  
21 194. The construct of claim 182, wherein the population of cells is cultured *in vitro* before the  
22 decellularized tissue engineered construct is seeded therewith.

23  
24 195. The construct of claim 182, wherein the population of cells is selected from the group  
25 consisting of: smooth muscle cells, cardiac muscle cells, epithelial cells, endothelial cells,  
26 urothelial cells, fibroblasts, myoblasts, chondrocytes, chondroblasts, osteoblasts, osteoclasts,  
27 hepatocytes, bile duct cells, pancreatic islet cells, thyroid, parathyroid, adrenal, hypothalamic,  
28 pituitary, ovarian, testicular, salivary gland cells, adipocytes, and precursor cells.

1 196. The construct of claim 182, wherein the population of cells comprises cells of at least two  
2 different cell types.

3  
4 197. The construct of claim 182, wherein the population of cells comprises neonatal cells.

5  
6 198. The construct of claim 182, wherein the population of cells comprises human cells.

7  
8 199. The construct of claim 182, wherein the decellularized tissue engineered construct is  
9 maintained for growth period under growth conditions suitable for the growth of the population  
10 of cells.

11  
12 200. The construct of claim 182, wherein the decellularized tissue engineered construct  
13 comprises a decellularized tissue engineered construct that has been subjected to a mechanical  
14 force during a growth period.

15  
16 201. The construct of claim 182, wherein the decellularized tissue engineered construct  
17 comprises a decellularized tissue engineered construct that has been subjected to a pulsatile  
18 stimulus during a growth period.

19  
20 202. The construct of claim 182, wherein the decellularized tissue engineered construct  
21 comprises a decellularized construct that has been subjected to electrical stimulation during a  
22 growth period.

23  
24 203. The construct of claim 182, wherein the decellularized tissue engineered construct  
25 comprises a decellularized tissue engineered construct that has been treated with a growth factor  
26 during a growth period.

27  
28 204. The construct of claim 182, wherein the decellularized tissue engineered construct  
29 comprises a decellularized tissue engineered construct that has been exposed to serum during a  
30 growth period.



1  
2 205. A method for treating a subject suffering from tissue damage or loss comprising:  
3       implanting the construct of claim 182 into a subject in need thereof.  
4

5 206. The method of claim 205, further comprising the step of:  
6       treating the construct with a biologically active agent before the implanting step, whereby  
7 the construct serves as a vehicle for delivery of the biologically active agent to the subject.  
8

9 207. The method of claim 205, further comprising the step of:  
10       treating the construct with a biologically active agent before the implanting step, whereby  
11 the biologically active agent is selected to enhance recellularization or vascularization of the  
12 construct after the implanting step.  
13

14 208. The method of claim 205, wherein the biologically active agent comprises a pharmaceutical  
15 composition.  
16

17 209. The method of claim 205, wherein the biologically active agent is selected from the group  
18 consisting of: growth factors, adhesion factors, soluble extracellular matrix proteins,  
19 thrombomodulators, antibiotics, and agents that augment hemocompatibility.  
20

21 210. The method of claim 205, wherein the implanting step comprises supplementing or  
22 replacing a blood vessel of the subject.  
23

24 211. The method of claim 205, wherein the implanting step comprises supplementing or  
25 replacing a tissue of the subject, the tissue selected from the list consisting of: a heart valve, a  
26 muscle, a joint, a ligament, a tendon, a bone, and an organ.  
27

28 212. A method for treating a subject suffering from tissue damage or loss comprising:  
29       implanting the construct of claim 199 into a subject in need thereof.  
30

1 213. The method of claim 212, further comprising the step of:

2 treating the construct with a biologically active agent before the implanting step, whereby  
3 the construct serves as a vehicle for delivery of the biologically active agent to the subject.  
4

5 214. The method of claim 212, further comprising the step of:

6 treating the construct with a biologically active agent before the implanting step, whereby  
7 the biologically active agent is selected to enhance recellularization or vascularization of the  
8 construct after the implanting step.  
9

10 215. The method of claim 212, wherein the biologically active agent comprises a pharmaceutical  
11 composition.  
12

13 216. The method of claim 212, wherein the biologically active agent is selected from the group  
14 consisting of: growth factors, adhesion factors, soluble extracellular matrix proteins,  
15 thrombomodulators, antibiotics, and agents that augment hemocompatibility.  
16

17 217. The method of claim 212, wherein the implanting step comprises supplementing or  
18 replacing a blood vessel of the subject.  
19

20 218. The method of claim 212, wherein the implanting step comprises supplementing or  
21 replacing a tissue of the subject, the tissue selected from the list consisting of: a heart valve, a  
22 muscle, a joint, a ligament, a tendon, a bone, and an organ.  
23